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AGRICULTURAL RESEARCH ADMINISTRATION

Bureau of Agricultural and Industrial Chemistry
Agricultural Research Center
Beltsville, Maryland

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TO: G. W. Irving, Jr., Assistant Chief,
BAIC, South Building, Washington, D. C.

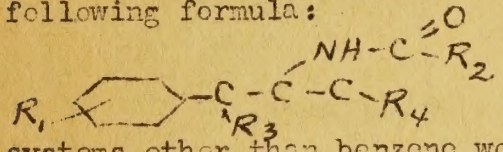
FROM: T. D. Fontaine, Head, Biologically Active
Compounds Div., ARC, Beltsville, Maryland

SUBJECT: Report of trip to the University of Notre Dame, June 15-17, 1950 for the purpose of attending the Second National Medicinal Chemistry Symposium of the American Chemical Society; to Mallinckrodt Chemical Works, St. Louis, Mo., June 19, 1950 for the purpose of conferring with Dr. V. H. Wallingford and associates on tomatine and tomatidine.

Summary: - The Medicinal Chemistry Symposium.

1. Drugs Reducing Skeletal Muscle Tone. - These drugs are naturally-occurring alkaloids, which have been divided into four structural types: (a) Berberine, (b) Coclaurine, (c) Bisecoclaurine, and (d) quaternary alkaloids related to curine, and several synthetic compounds of the hydroxyquinoline type.

2. Antibiotics. - (a) Streptothricin. The chemistry of an antibiotic which at the present time has no medicinal application is presented. (b) Chloromycetin. Analogues of chloromycetin are described. Substitutions have been made in four positions in the molecules as represented by the Rs in the following formula:



None of the analogues of chloromycetin reported at this meeting were as active as chloromycetin. It was intimated that perhaps compounds containing ring systems other than benzene were active but because of pending patent action they were not discussed.

3. Chemistry of Cortisone and ACTH. - (a) Cortisone. An excellent review of the chemistry involved in the determination of the structure and the synthesis of cortisone is presented. It was mentioned that Dr. Elderfield's group has synthesized a compound having cortisone-like activity. (b) ACTH. Particular attention should be given to this topic. Dr. E. E. Hays, Armour Research Institute, takes Dr. Li's work apart. Dr. Li was unable to present his paper but the abstract of his paper will be found in the abstracts of the Symposium.

4. Radioactive and Stable Isotopes in Biochemical and Pharmaceutical Investigations. - The complete speech on the use of radioactive isotopes is attached and its reading is recommended. The use of stable isotopes is an older story but none the less interesting.

5. Cardiac Drugs.- The isolation and structure determination of the active principle in red squill is reported. It is a cardiac glycoside, scilliroside. Its aglycone, scillirosidin, is the most powerful cardiac agent discovered to date. The importance of enzymes for removing glycosidal portions of cardiac drugs is emphasized.

Mallinckrodt Chemical Works. - This company has the facilities and the necessary background knowledge to produce alkaloids from plants.

Report:

The program of the Second National Medicinal Chemistry Symposium of the American Chemical Society held at the University of Notre Dame, June 15-17, 1950 was as follows:

The Pharmacology of Drugs Reducing Skeletal Muscle Tone
A. R. McIntyre
Some Aspects of the Chemistry of Drugs Reducing Skeletal Muscle Tone
D. S. Tarbell
Some Aspects of Antibiotic Research
W. R. Taylor
Analogues of Chloromycetin
H. M. Crooks, Jr.
The Needle in the Haystack
E. H. Volwiler
The Chemistry of Cortisone
H. L. Mason
The Chemistry of Adrenocorticotropic Hormone (ACTH)
E. E. Hays (Originally scheduled for presentation by C. H. Li)
The Use of Radioactive Isotopes in Pharmaceutical Studies
D. L. Tabern
The Use of Heavy Isotopes in Biochemical Investigations
G. H. Hitchings (Originally scheduled for presentation by G. B. Brown)
Some Chemical Aspects of Cardiac Drugs
A. Stoll
Pharmacological and Clinical Aspects of Cardiac Drugs
R. Wegria

I was fortunate in being able to obtain 5 copies of the Abstracts of this program for distribution to each Regional Research Laboratory and to the Washington Office. Since the abstracts are excellent I shall refer readers to them for additional information. There were two changes in the program due to the illness of Dr. Li and Dr. Brown. The substitute speakers did an admirable job and brought out different viewpoints, in one case almost directly opposite to the abstract.

Dr. Volwiler's talk, "The Needle in the Haystack", was the after dinner speech which he stated should be titled "The Lucky Chemist" but in either case his talk would have been the same. Dr. Volwiler reviewed the lucky breaks and accidents which have resulted in many of the outstanding chemical advances. His main point was that a keen observer has been responsible for these major accomplishments.

I did not attend the second paper "Some Aspects of the Chemistry of Drugs Reducing Skeletal Muscle Tone" but took that time to visit the Laboratories of Bacteriology, University of Notre Dame (Lobund).

The last paper of the program "Pharmacological and Clinical Aspects of Cardiac Drugs" is not abstracted in the printed abstracts and because of the restricted phase of the subject discussed I obtained very little information. Dr. Wegria's talk, while I am sure it was to the point, was difficult to follow because of his accent. He restricted

his remarks to coronary flow and drugs such as epinephrin, nicotine, etc. which increase coronary flow, and other compounds such as nitroglycerine, nitrites, aminophllin, procaine and nematol, which decrease coronary flow.

Analogues of Chloromycetin, by H. M. Crooks, Jr., Parke, Davis & Co. - This was an excellent paper, with two exceptions. It was obvious that due to patent actions pending much good material was omitted and this report was primarily of negative results, and second, the activities of the compounds reported were caged in general terms. In general, most of the compounds listed in the abstract had little or no activity or activity in the range of 20% of that found for chloromycetin. Dr. Crooks very carefully avoided any discussion of chloromycetins synthesized with rings other than benzene but indicated they had been prepared and were awaiting patent action. The compounds having a threo configuration are active but those with erythro configuration are inactive. Chloromycetin is only about 1/8th as active against M. tuberculosis as streptomycin and the para chloro, bromo, and iodo substituted compounds are only about 1/8th as active as chloromycetin.

Chloromycetin is active against the larger viruses so there is a need for antibiotics active against the smaller viruses. Chloromycetin apparently affects the bacterial esterase enzyme system but has no effect on the protein and carbohydrate enzyme systems. About 90-92% of chloromycetin is excreted in the urine as the glucuronide, 6% comes through unchanged, and a small amount is reduced by the bacteria. Dr. Crooks, in answer to one question, stated that aureomycin and chloromycetin are different compounds but that the antibiotic spectrum and ultraviolet absorption spectrum of terramycin closely approximate that of chloromycetin. Because the New York Academy of Sciences was holding a three day conference on Terramycin concurrently with this meeting Dr. Peck, Merck and Co., was given an abstract to read should there be a question of the similarity of chloromycetin and terramycin. His report is as follows and it is apparent that the two compounds are not identical.

Terramycin forms a hydrochloride, chloromycetin does not. The empirical formula for terramycin is $C_{22}H_{24-26}N_2O_9 \cdot 2H_2O$. Terramycin melts at 185°C and has $[\alpha]_D^{25}$ 196° in 1% 0.1N HCl; gives positive ferric chloride, Friedel-Craft and Molisch tests; sodium and potassium salts are crystalline.

The Chemistry of Cortisone, by H. L. Mason, Mayo Foundation - The abstract of this paper summarizes the important reactions involved in the establishment of structure and partial synthesis of cortisone. It was reported that the total synthesis of cortisone was being attempted by at least 6 major laboratories and with some degree of success. For example, Sir Robert Robinson's laboratory in England has succeeded in the synthesis of the first three rings but in adding the fourth ring a total of 64 isomers are possible. If anyone could provide a reagent

to replace osmium tetraoxide (OsO_4) for the introduction of the 17-hydroxy group, it was felt that work would progress more rapidly.

The mechanism of action of cortisone was discussed and at least four things result from the administration of cortisone. Cortisone (1) prevents the spread of hyaluronidase, (2) is a growth inhibitory substance, (3) inhibits the system which produces histamine and thus lessen symptoms of allergy, (4) inhibits inflamatory action. Cortisone is most effective in carbohydrate metabolism but poorest for controlling mineral retention.

Dr. Byron Riegel, North Western University, has fed small animals carbon labeled progesterone. In mice, progesterone was found in the adrenal gland whereas in rats none was found. The pituritary gland of both animals were found to contain a large amount of progesterone but most was found in the liver. Eventually all of the carbon labelled compound is excreted in the fecal matter. In none of these experiments was progesterone actually isolated and what they measured was the non-saponifiable fraction which they assumed to be the original carbon labeled progesterone.

It was further reported that Compound F (17-OH corticosterone) is no more active than cortisone in the treatment of arthritis. Dr. Elderfield, Columbia University, indicated at lunch one day that government patent attorneys have just filed patents on the preparation of at least one of the compounds synthesized by his group which has been found to have cortisone-like activity.

The Chemistry of Adrenocorticotropic Hormone (ACTH), by E. E. Hays, Armour Research Institute - The abstract of the paper entitled "The Chemistry of Adrenocorticotropic Hormone (ACTH)", by C. H. Li, should be critically evaluated. Dr. E. E. Hays, Armour Research Institute delivered the paper due to Dr. Li's illness in Chicago. Dr. Li had delivered a speech at the dedication of the Goldblatt Memorial Cancer Hospital the day before and from informal comments circulated by people at the meeting Dr. Li's illness was not unusual when he has to back up his conclusions before a scientific group. Dr. Hays pulled no punches in his talk.

Dr. Hays stated there was much misinformation available on ACTH, based on too little chemistry and too impure material. He stated that most of Dr. Li's work can be disregarded. Dr. Li has never isolated ACTH or the active peptide although he reported it in 1942-43. Dr. Li's work was based only on electrophoretic data; no solubility data were obtained; ultracentrifuge data has since shown a multiple component product. Armour's standard, La-1-A, has greater activity than the "pure ACTH" of Dr. Li's.

ACTH is stable in boiling dilute acid but not at pH 7 or in alkali. Dialysis is a poor criteria for molecular size; trichloroacetic acid is of no value either.

Dr. Hays presented the following information. Pork is the only reasonable source of ACTH. The material behaves as if its molecular weight is 10,000 instead of 1,000. This figure is based on the recovery of 92% of the ACTH activity of a preparation in an ultracentrifuge. The centrifuge was operated at 6,000 r.p.m. and under these conditions compounds of 10,000 or greater molecular weight are collected in the lower half of the cell. By distribution techniques they have succeeded in preparing ACTH material 120 times as active as their standard, La-1-A. This product does not give a ninhydrin reaction. Twelve amino acids have b en identified in the hydrolysate of the material assaying 120 times the standard, La-1-A. Activity is destroyed by trypin but not by pepsin.

It is Dr. Hays opinion that Dr. Li's preparation of "pure ACTH" is only 0.5% pure, thus his conclusions have been based on material containing 99+% impurities.

Radio Isotopes - Their Use In Pharmaceutical and Medical Studies, by D. L. Tabern, J. D. Taylor and G. I. Gleason, Abbott Laboratories - This talk was well presented and will be of particular interest to the Laboratories using radioactive isotopes or those who contemplate their use. A mimeographed copy of his complete talk is attached and additional copies may be obtained by writing to Dr. Tabern, Abbott Laboratories, North Chicago, Illinois. Phases of work reported on are as follows:

1. Abbott Isotope Program
2. Uses of P-32
3. Uses of I-131
4. Tumor Localization
5. Other Diagnostic Methods
6. Tumor Therapy
7. Barbiturates and Thiobarbiturates
8. Sulfur Amino Acids
9. The Dynamic State
10. Membrane Permeability
11. Antigens and Antibodies
12. Fat Metabolism
13. Thyroid and Anti Thyroid
14. Drug Metabolism
15. Isotopes As An Analytical Tool
16. Mechanical Tracing
17. Toxicity of C-14
18. Metabolic Studies with P-32
19. Pharmaceutical Use of Iron 55-59

Dr. Tabern reports that four great obstacles to the full use of isotopes in chemistry, pharmacy and medicine are being dissipated.

"(1) Research workers in general are rapidly loosing their fear of isotopes, and coming to look upon them as a new and very useful tool to be applied whenever their employment promises to lead to useful information, or the solution of specific problems.

(2) A well trained group of "isotopologists" is gradually being built up who understand the limitations and hazards, as well as the advantages of isotopic studies.

(3) Basic data, methods, and equipment are being developed to the point where one can proceed with a problem without spending months in preliminary work.

(4) The availability of tagged compounds, reagents, and "research medicinals" of a purity and pharmaceutical quality equal or superior to ordinary drugs is well on the way to being an established fact."

Dr. Taborn described a method, developed at Abbott Laboratories, for measuring absolute β counts. It is quite simple. Only the sensitive portion of the tube window is used. A shield with a single beveled hole in it is placed in front of the window so that only a small center portion of the tubes' window is used. This method works well with helium-alcohol tubes but not with some others. From the geometry of the tube and the distance of the source emitter the absolute β count can be calculated. He showed comparative values for samples measured by this method and by Oak Ridge, where another method was used, and there was excellent agreement.

The Use of Heavy Isotopes in Biochemical Investigations, by G. B. Brown, Sloan-Kettering Institute for Cancer Research - Dr. G. B. Brown became ill with the mumps and was unable to deliver his talk, but his abstract is so complete that those interested will find a good summary in the abstracts. Dr. G. H. Hitchings, Burroughs Wellcome and Co., reviewed some of the work in this field.

Some Chemical Aspects of the Cardiac Glycosides, by A. Stoll, Sandoz, Inc. - Dr. A. Stoll's talk was of considerable interest. Characteristic properties of all the cardiac glycosides are (1) a five or six-membered unsaturated lactone ring in position 17 of the aglycone, (2) a hydroxyl group, which is coupled with the sugar residue, situated at position 3, (3) a tertiary hydroxyl group at position 14, and (4) other substituents in the aglycone skeleton may include further hydroxyl groups and aldehyde groups.

The elucidation of the structure of the active principle of red squill was a major contribution of this paper. A previously unknown cardiac glycoside, scilliroside, which proved to be an extremely powerful rat poison besides possessing a powerful action on the heart was discussed. It has been determined that the monosides are more powerful than the polysides as cardiac drugs and that the monosides, with only two exceptions thus far, are more powerful than the aglycones.

The importance of enzymes in producing monosides and aglycones was illustrated. Until very recently, it had not been possible to split the linkage between the aglycone and the sugar chain by enzymatic means. By the use of enzyme systems present in seeds of Coronilla glauca it has been possible for the first time to prepare scillirosidin, the aglycone of scilliroside. Scillirosidin has proven to be the most powerful cardiac agent obtained to date.

In another instance, they have succeeded in isolating a previously unknown main genuine glycoside, k-strophanthoside, from the seeds of Strophanthus kombe. By the use of α-glycosidase they were able to show that the terminal glucose is α-glucosidal and by the use of strophanthiobiase to show that the glucose molecule nearer the aglycone is β-glycosidal. α- and β-glucosidal linkages in the same molecule are very rare.

Dr. John W. Chermodra, Merck and Co., introduced an interesting item into the discussion. In view of the structural requirements given for a cardiac agent, Dr. Chermodra stated that it is interesting to note that flurcacetic acid gave the same effects as those reported for cardiac glycosides. This observation was of considerable interest but apparently others present had not worked sufficiently with this compound to continue the discussion.

Laboratories of Bacteriology, University of Notre Dame, or Lobund as it is called - This laboratory was organized for basic research in the biological and medical sciences. The organization consists of three Divisions.

Lobund Organization

James A. Reyniers, Director
Philip C. Trexler, Assistant Director
Robert F. Ervin, Administrative Director

Germ-Free Life Division

Anatomy and Physiology
Dr. Helmut A. Gordon, Physiologist
Bacteriology and Serology
Morris Wagner, Bacteriologist
Biochemistry and Nutrition
Dr. Thomas D. Luckey, Biochemist
Germ-Free Life Production
Bernard A. Teah

Biological Engineering Division

Arthur W. Phillips, Biological Engineer

Microrgy Division

Philip C. Trexler, Bacteriologist

The formal dedication of this laboratory is to take place on June 21, 1950. Dr. Luckey conducted the groups through the laboratory and explained its operation. Only the small units were in operation for the rearing of germ-free animals; the large unit with its elaborate system of maintaining sterile conditions will be in operation soon. Thus far, work has been concentrated on the development of methods and techniques for the rearing of germ-free animals in quantities large

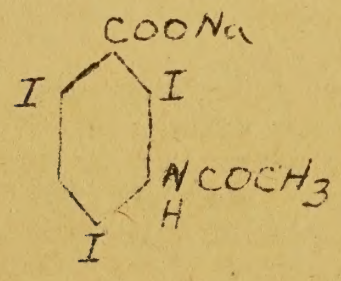
enough to be used on a wide variety of problems. Since all food must be sterile, a considerable number of problems have arisen here. Germ-free Bantam chickens have been relatively easy to hatch and study. On the other hand, rats present more of a problem. They have obtained reproduction from germ-free rats but in every case, thus far, the young have been unable or unwilling to nurse although the mother rat produces an ample supply of milk. They must be fed by medicine droppers. Earlier predictions, that a presumably non-pathogenic organism would be pathogenic to germ-free animals has been borne out.

Dr. Carl T. Bahner, Carson-Newman College, Jefferson City, Tennessee - Dr. Bahner has been synthesizing quaternary ammonium compounds to be tested in cancer investigations. A number of them might have plant-growth regulating activity. He agreed to send us several compounds. Dr. Irving, Markely, and others may remember Dr. Bahner (pronounced Benner) from his visit to the Southern Laboratory before 1945. He was then consultant for TVA.

Mallinckrodt Chemical Works, St. Louis, Mo., June 19 - This company has been particularly interested in tomatine and tomatidine. One of their representatives, Dr. Venerable, attended the Federation Meetings in Atlantic City, N. J. and passed along the information on tomatine and tomatidine which I had presented. In a short time Dr. Melvin A. Thorpe, In Charge, Formulation and Product Development, visited the Washington office. I learned that Dr. A. H. Homeyer, Associate Director of Organic Research, is responsible for their interest in this subject. Unfortunately, he was out of town during my visit.

I reviewed the published and previously presented work on tomatine and tomatidine with Dr. V. H. Wallingford, Director of Organic Research and Development, and Drs. Thorpe and Venerable. As it turns out, they are particularly interested in the preparation of these materials or their degradation products should they have either chemical or medicinal possibilities. Their interests arise from the fact that they are a major producer of opium alkaloids and many other alkaloids, but do not market them directly. They are, therefore, quite capable of handling this type of work.

Dr. Wallingford takes an active part in the research work and spends some time in the laboratory each day. He has been instrumental in the development of Urokon Sodium, a 30% solution of the sodium salt of 3-acetylamino-2,4,6-triiodobenzoic acid, for intravenous and retrograde urography. It is claimed that this material is among the least toxic materials in general use as contrast media for urography. This compound may be of interest in plant-growth regulator investigations.



Conclusions and Recommendations: - This Medicinal Chemical Symposium has served to emphasize the possible medicinal value of any biologically active compound isolated from plants. Alkaloids which were once believed to be too toxic for use are now finding their place in medicine. Likewise, cardiac glycosides, while poisonous, are finding more outlets.

While it has been demonstrated that an antibiotic can be synthesized, once it has been isolated from its natural source, the specific chemical groups and the spatial relationship of these groups are very important. It appears that, at the present, the production of antibiotics from natural sources is the best method.

The general feeling is that the production of cortisone by synthetic methods will soon make it possible to supply the demand. In the case of ACTH, the chemical synthesis appears to be almost impossible if the molecular weight is of the order of 10,000. Much misinformation concerning the chemistry of ACTH was corrected at this meeting.

Radioactive and stable isotopes have solved many perplexing problems. However, many concepts of the mode of action of drugs have had to be revised since a specific drug does not necessarily concentrate at the point where it exerts its action.

It is recommended that the Bureau send at least one representative to meetings of this type to collect information which will be useful to the Bureau's scientific personnel.

Mallinckrodt Chemical Works has the facilities and the necessary background knowledge to produce alkaloids, such as tomatine or its aglycone, tomatidine.

Thomas A. Fontaine

Attachments - 2

14 - Washington Office
1 - Eastern Laboratory
1 - Western Laboratory
1 - Northern Laboratory
1 - Southern Laboratory
10 - BACD Files